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Supplemental information

Long-term maintenance of dystrophin expression and resistance to injury of skeletal muscle in gene edited DMD mice

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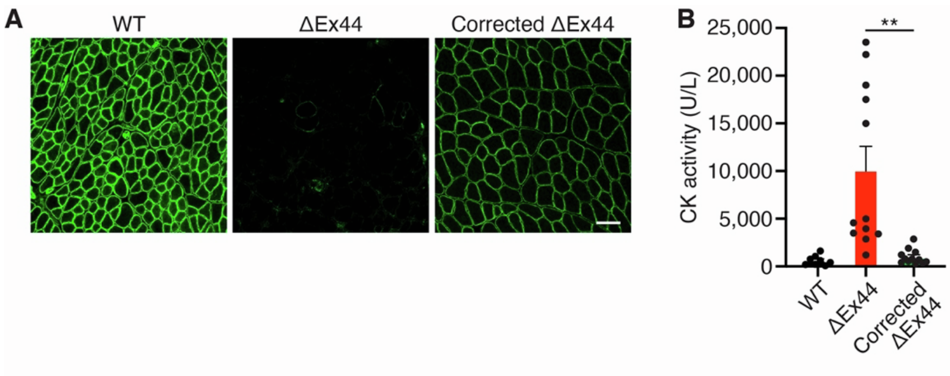

Figure S1. Verification of correction of ΔEx44 mice. (A) Immunohistochemistry shows dystrophin restoration in the tibialis anterior muscle of corrected ΔEx44 mice. Dystrophin is shown in green. Scale bar, 100 μm (n = 3). (B) Serum creatine kinase (CK) activity in 4-week-old WT, ΔEx44, and corrected ΔEx44 mice. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. **$P<0.005$ ($n \geq 9$).

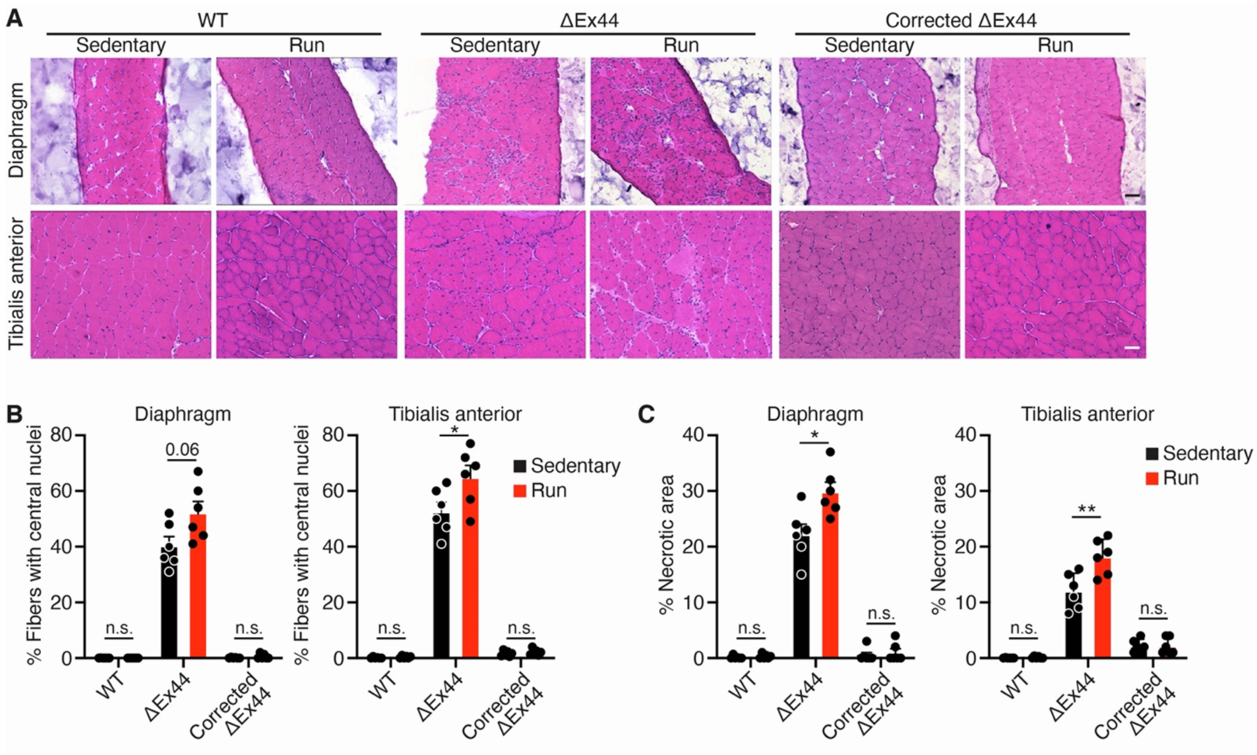

Figure S2. CRISPR/Cas9 genome editing prevents skeletal muscle injury induced by downhill running in the diaphragm and tibialis anterior muscles of corrected ΔEx44 mice. (A) H&E staining of the tibialis anterior and diaphragm muscles from WT, ΔEx44, and corrected ΔEx44 mice that were either sedentary or run downhill for 4 weeks. Scale bar, 100 µm. (B) Quantification of centralized nuclei in diaphragm and tibialis anterior muscles from sedentary or downhill run WT, ΔEx44, and corrected ΔEx44 mice. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. *P<0.05 (n = 6). (C) Quantification of necrotic area in diaphragm and tibialis anterior muscles from sedentary or downhill run WT, ΔEx44, and corrected ΔEx44 mice. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. *P<0.05, **P<0.005 (n = 6).

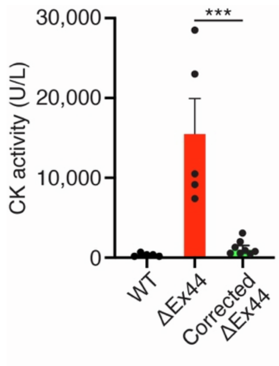

Figure S3. Serum creatine kinase (CK) activity in WT, ΔEx44, and corrected ΔEx44 mice following 4 weeks of downhill running. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. ***P<0.001 (n ≥ 5).

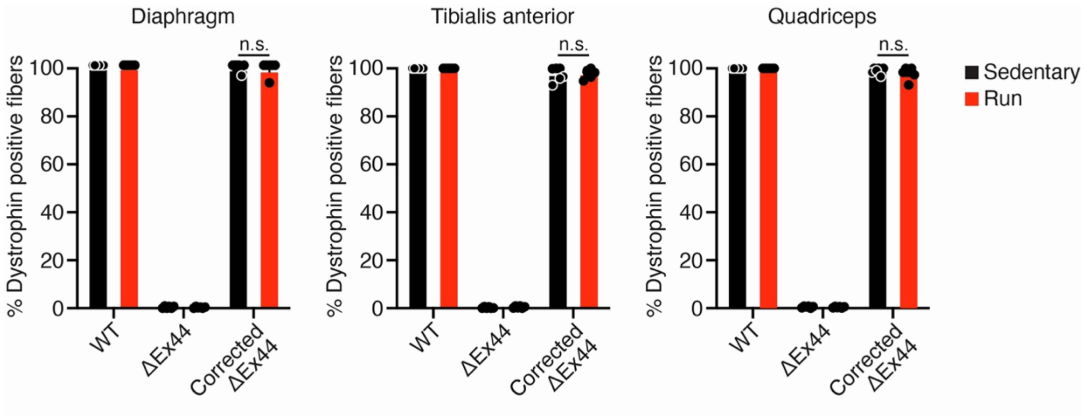

Figure S4. Corrected ΔEx44 mice retain dystrophin positive fibers in diaphragm, tibialis anterior, and quadricep muscles following 4 weeks of downhill running. Data are shown as mean ± SEM. Unpaired Student's t-test was performed (n = 6).

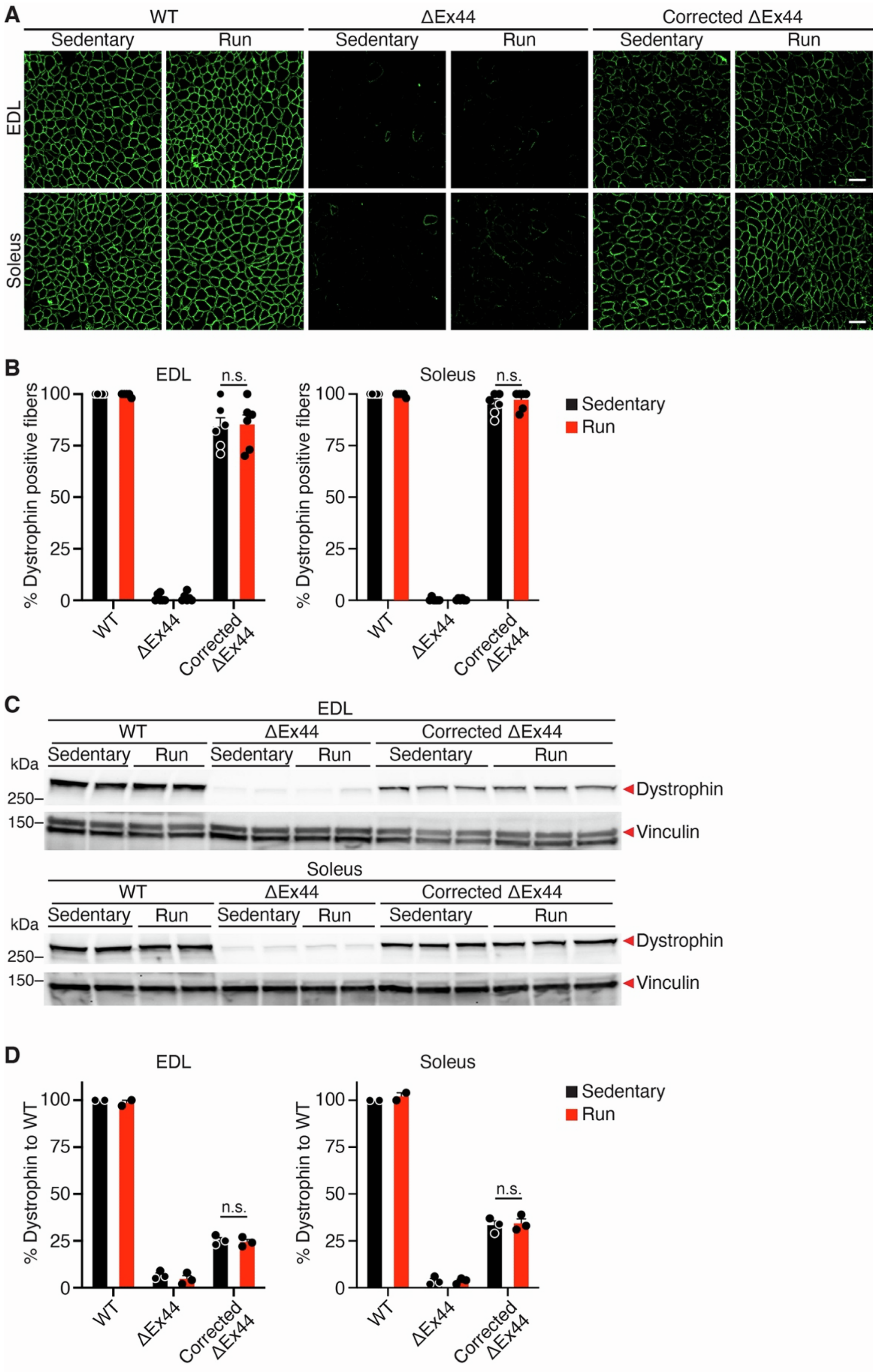
A
WT
ΔEx44
Corrected ΔEx44
Sedentary
Run
Sedentary
Run
Sedentary
Run
EDL
Soleus
B
EDL
Soleus
n.s.
n.s.
% Dystrophin positive fibers
100
75
50
25
0
WT
ΔEx44
Corrected ΔEx44
Sedentary
Run
C
EDL
WT
ΔEx44
Corrected ΔEx44
Sedentary
Run
Sedentary
Run
Sedentary
Run
kDa
250–
150–
Dystrophin
Vinculin
Soleus
WT
ΔEx44
Corrected ΔEx44
Sedentary
Run
Sedentary
Run
Sedentary
Run
kDa
250–
150–
Dystrophin
Vinculin
D
EDL
Soleus
% Dystrophin to WT
100
75
50
25
0
n.s.
n.s.
WT
ΔEx44
Corrected ΔEx44
Sedentary
Run

Figure S5. Corrected ΔEx44 mice retain dystrophin positive fibers in the EDL and soleus muscles following 4 weeks of downhill running. (A) Immunohistochemistry shows retention of dystrophin positive fibers in the EDL and soleus muscles in corrected ΔEx44 mice following 4 weeks of downhill running. Dystrophin is shown in green. Scale bar, 100 μm. (B) Quantification of the percentage of dystrophin positive fibers in EDL and soleus muscles. Data are shown as mean ± SEM. Unpaired Student's t-test was performed ($n \geq 5$). (C) Western blot analysis shows retention of dystrophin protein in the EDL and soleus muscles of corrected ΔEx44 mice following 4 weeks of downhill running. Vinculin was loading control. (D) Quantification of dystrophin protein in EDL and soleus. Dystrophin protein levels were first normalized to vinculin and then to WT sedentary controls. Data are shown as mean ± SEM. Unpaired Student's t-test was performed ($n = 3$).

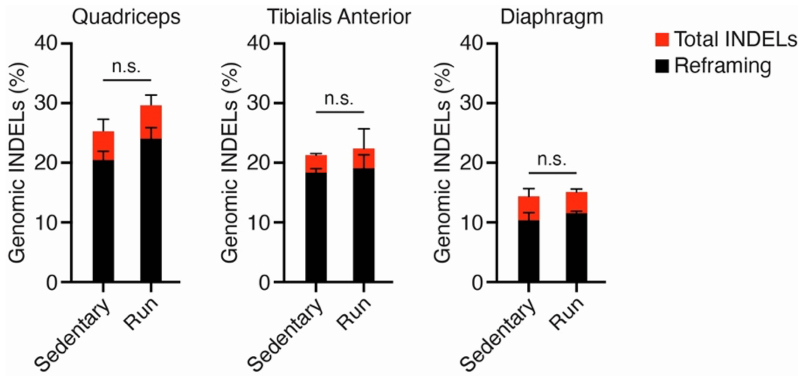

Figure S6. INDEL frequency at the sgRNA target site in corrected ΔEx44 mice does not change with downhill running. Reframing refers to INDELs (+1 or -2 nucleotide(s)) that reframe exon 45 and restore the dystrophin open reading frame. Data are shown as mean ± SEM. Unpaired Student's t-test was performed (n = 3).

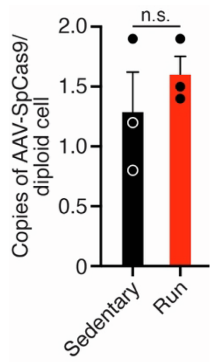

Figure S7. Corrected ΔEx44 mice retain AAV-SpCas9 viral genomes in the quadriceps muscle following 4 weeks of downhill running. Data are shown as mean ± SEM. Unpaired Student's t-test was performed (n = 3).

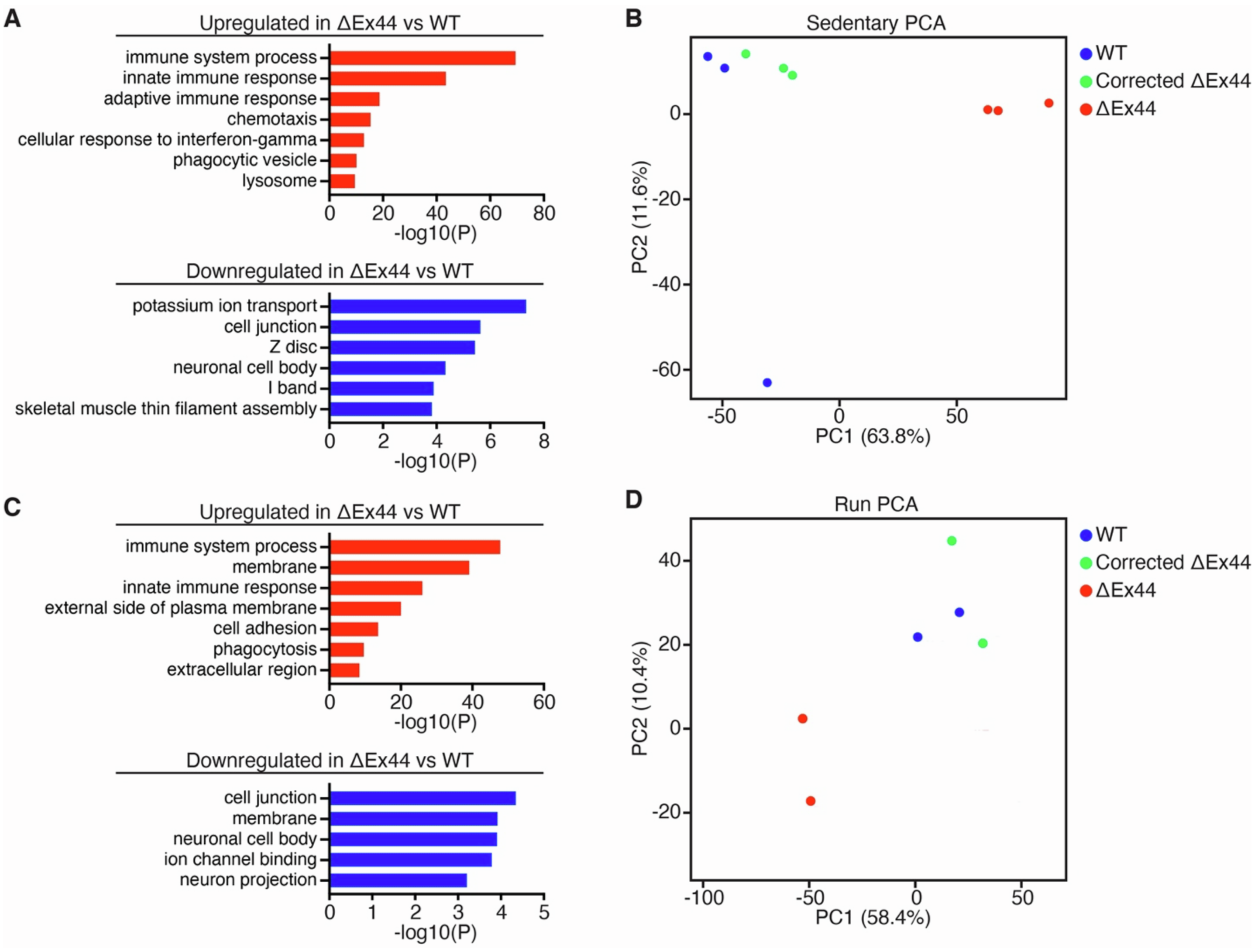

Figure S8. Additional transcriptional comparisons between WT, ΔEx44, and corrected ΔEx44 before and after downhill running. (A) Selected GO terms of up- and down-regulated genes in ΔEx44 quadriceps muscle relative to WT. (B) Principal component analysis of transcriptomes from WT, ΔEx44, and corrected ΔEx44 quadriceps muscle. (C) Selected GO terms of up- and down-regulated genes in downhill run ΔEx44 quadriceps muscle relative to WT. (D) Principal component analysis of transcriptomes from WT, ΔEx44, and corrected ΔEx44 quadriceps muscles following 4 weeks of downhill running.

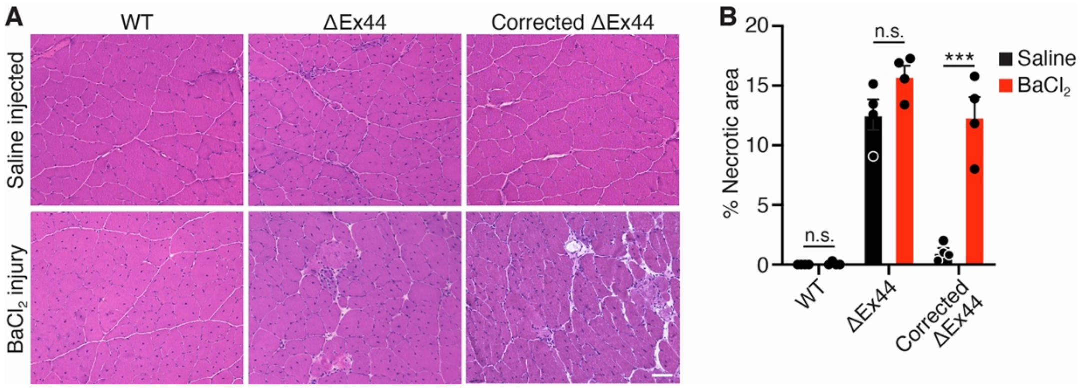

Figure S9. Corrected ΔEx44 tibialis anterior muscle exhibits muscle necrosis two months following acute injury induced by $BaCl_2$. (A) H&E staining of WT, ΔEx44, and corrected ΔEx44 tibialis anterior muscles two months following $BaCl_2$ or saline injection. Scale bar, 100 μm. (B) Quantification of necrosis in WT, ΔEx44, and corrected ΔEx44 tibialis anterior muscles two months following $BaCl_2$ or saline injection. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. ***$P<0.001$ ($n = 4$).

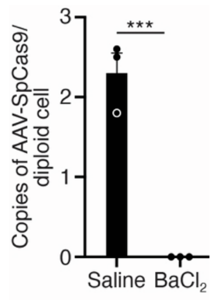

Figure S10. AAV-SpCas9 viral genomes are lost following $BaCl_2$ induced injury. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. ***P<0.001 (n = 3).

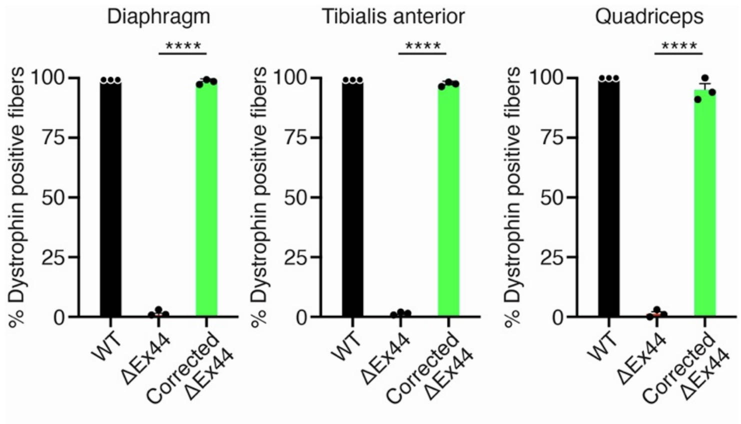

Figure S11. 18-month-old corrected ΔEx44 mice retain dystrophin positive fibers in diaphragm, tibialis anterior and quadriceps muscles. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. ****$P<0.0001$ (n=3).

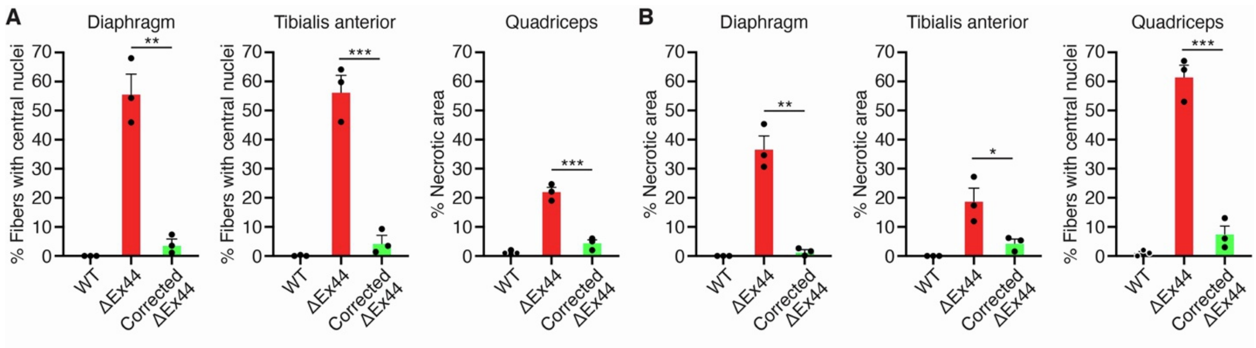

Figure S12. 18-month-old corrected ΔEx44 mice exhibit significantly reduced histological markers of muscle degeneration. (A) Quantification of centralized nuclei in the diaphragm, tibialis anterior and quadriceps muscles from 18-month-old WT, ΔEx44, and corrected ΔEx44 mice. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. **P<0.005, ***P<0.001 (n = 3). (B) Quantification of necrosis in the diaphragm, tibialis anterior and quadriceps muscles from 18-month-old WT, ΔEx44, and corrected ΔEx44 mice. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. *P<0.05, **P<0.005, ***P<0.001 (n = 3).

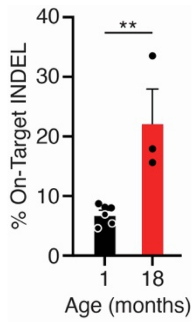

Figure S13. On-target INDEL comparison between 4-week-old and 18-month-old corrected ΔEx44 tibialis anterior muscle. Data are shown as mean ± SEM. Unpaired Student's t-test was performed. **P<0.005 (n ≥ 3).

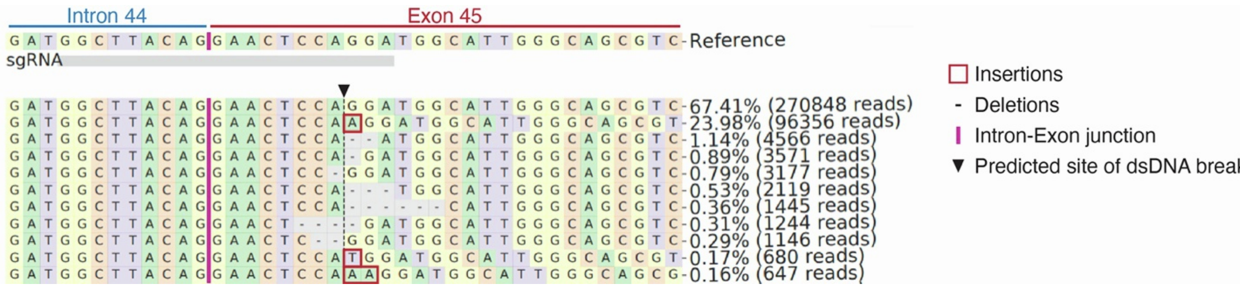

Figure S14. Genomic edits at the on-target site in 18-month-old corrected ΔEx44 tibialis anterior muscles.

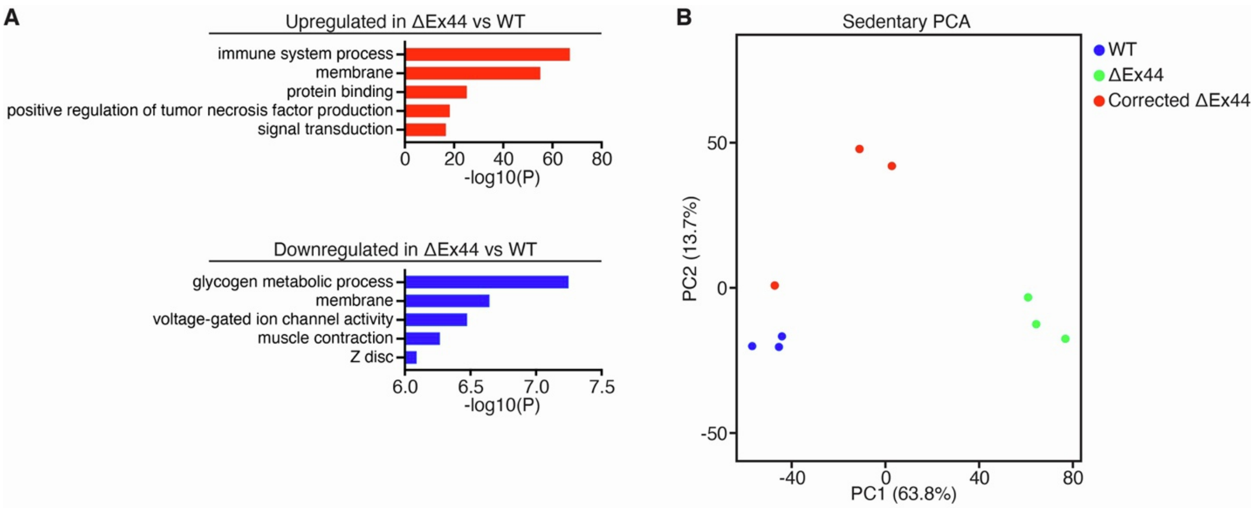

Figure S15. Additional transcriptional comparisons between 18-month-old WT, ΔEx44, and corrected ΔEx44 quadriceps muscles. (A) Selected GO terms of up- and down-regulated genes in 18-month-old ΔEx44 quadriceps relative to WT. (B) Principal component analysis of transcriptomes from 18-month-old WT, ΔEx44, and corrected ΔEx44 quadriceps muscle.

Table S1. Amplicon-based deep sequencing of 18-month-old corrected ΔEx44 tibialis anterior muscle.

Sample ID	Total Editing %	Total Reframing %	Most Common Reframing Events		% AAV Integration at Target Site
			+1 nt %	-2 nt %	
Corrected ΔEx44 TA #1	18.35	15.9	14.15	1.19	0.14
Corrected ΔEx44 TA #2	21.25	17.96	16.95	0.87	0.37
Corrected ΔEx44 TA #3	31.1	26.42	24.28	1.47	0.47

Table S2. Primers used for AAV-SpCas9 copy number analysis and for on-target TIDE.

Experiment Name	Primer Name	Primer Sequence
AAV-SpCas9 Copy Quantification	SpCas9-F	TGAAAGAGGACTACTTCAAGAAAATC
	SpCas9-R	TTGTCCTTGATAATTTTCAGCAGATC
TIDE Analysis of On-target Editing	mEx45-TIDE-F	CCCTGAGCTGAAGTGAGAGG
	mEx45-TIDE-R	ACCTCTTTCTCCTTTCTGCCAG

Table S3. Primers for amplicon-based deep sequencing.

Primers for on and off-target amplicon-based deep sequencing			
Site	*Sequence (5'-3')*	*Product*	*miSeq Primers with Adapter*
ON-TARGET	CCCTGAGCTGAAGTGAGAGG	404	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCTGAGCTGAAGTGAGAGG
	ACCTCTTTCTCCTTTCTGCCAG		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACCTCTTTCTCCTTTCTGCCAG
OFF-TARGET 1	CTGCCCCAACAAGAGCATTCTAAG	374	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGCCCCAACAAGAGCATTCTAAG
	AGCCACTGTTTAACTTGCAGTCAC		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGCCACTGTTTAACTTGCAGTCAC
OFF-TARGET 2	CTTTCCTCCTCCACCCTCACAG	356	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTTCCTCCTCCACCCTCACAG
	TCCTGTTACATGTCCCCGACAC		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTGTTACATGTCCCCGACAC
OFF-TARGET 3	CTCAGAGAGTCGATGGAACTCCTG	442	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTCAGAGAGTCGATGGAACTCCTG
	TCCTATGGGGTCAATTTCTGCACA		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTATGGGGTCAATTTCTGCACA
OFF-TARGET 4	GGTTCTCAAAATGCCCTGTTGTGA	487	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTTCTCAAAATGCCCTGTTGTGA
	TCTCCTGGAGGGGTGAAAGAAAAG		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTCCTGGAGGGGTGAAAGAAAAG
OFF-TARGET 5	TGTGGGACTGCTAGAAAGTTTGGA	440	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGTGGGACTGCTAGAAAGTTTGGA
	GATCCCCGCCTGGAGTTTATTAGT		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATCCCCGCCTGGAGTTTATTAGT
OFF-TARGET 6	TGGACAAAGGAGCAAACAAAAGCT	413	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGGACAAAGGAGCAAACAAAAGCT
	TTTATGGACAGTTGAGGTGCCAGA		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTTATGGACAGTTGAGGTGCCAGA
OFF-TARGET 7	AAGGGACAGCTCAAAGACCTTCTT	398	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAGGGACAGCTCAAAGACCTTCTT
	ACTTCAAACGCACTGTCACATCAG		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTTCAAACGCACTGTCACATCAG
OFF-TARGET 8	TCTGAAGAAGCCCTTGGTCATTCA	459	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCTGAAGAAGCCCTTGGTCATTCA
	ATCCTCTACACGTAACAGGAAGCC		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCCTCTACACGTAACAGGAAGCC
OFF-TARGET 9	GAAGGCAGTCAAGCAGATTGGATC	414	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAGGCAGTCAAGCAGATTGGATC
	ACTAGCAGCCTTTGGATGAAGACA		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTAGCAGCCTTTGGATGAAGACA
OFF-TARGET 10	ATGACGACGACGACAATGTTGATG	445	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGACGACGACGACAATGTTGATG
	CCTCAAAGCCTTCTTGAAGGAAGC		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTCAAAGCCTTCTTGAAGGAAGC